Evaluation of anti-diarrheal activity in seed extracts of *Pongamia pinnata* (Fabaceae)

**ABSTRACT**

The purpose of the present study was to investigate scientifically the anti-diarrheal activity of seed extracts of *Pongamia Pinnata* by castor oil-induced diarrhea model in Wistar albino rats. Anti-diarrheal effect was evaluated by castor oil induced diarrhea. Loperamide 1mg/kg was used as standard drugs. Pet ether, ethanol, aqueous extract at doses 30 mg/kg, 250mg/kg, 100mg/kg respectively was investigated for anti-diarrheal activity in term of reduction in the rate of defecation in the rate of defecation in castor-oil induced diarrhea. In castor oil induced diarrhea, petroleum ether, ethanol and aqueous extract of *Pongamia pinnata* seeds significantly reduce the mean weight of faces when compared to standard drug. Experimental findings showed that ethanol extract of seeds of *Pongamia pinnata* possess significant anti-diarrheal activity as that of pet ether and aqueous extracts. Therefore it may be a potent source of anti-diarrheal drug in future.

**INTRODUCTION**

Diarrhea has been one of the most important health problems in developing countries (Sharma et al., 2010). Diarrhea is characterized by increased frequency of bowel movement, wet stool and abdominal pain. A majority of diarrheal cases are due to bacterial enteropathogens, diarrhoeagenic *Escherichia coli* (Safram et al., 2003). In developing countries they are the most common causes of morbidity and mortality. Many government and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 millin per year (Park, 2000). Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhea but they have some side effects. The natural drugs are used as antidiarrheal drugs, which are not always free from adverse effects (Hardman and Limberd, 1992). Therefore, the search for safe and more effective agents has an important area of active research. In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhea (Kouitcheu et al., 2006).

World Health Organization (WHO, 2004) has included a programme for the control of diarrhea, which involves the use of traditional herbal medicine. Several plants have been reported to be used in treating and managing diarrheal diseases. The plant *Pongamia pinnata* (Fabaceae). *Pongamia* is a medium sized glabrous tree popularly known as Karanja in Hindi, Indian beech tree in English and Ghanerakaran in Marathi. It is hardy and can survive in temperatures from 5 to 50 °C and altitudes from 0 to 1200m. *Pongamia* is native to a number of countries including India, Malaysia, Indonesia, Taiwan, Bangladesh, Sri Lanka and Myanmar. *Pongamia pinnata* possesses antiviral activity, antifungal and antibacterial activity, anti inflammatory activity, ulcer protective activity, and action against infectious diarrhea proved in leaf extract of plant (Salud et al., 2007 and Sangwan et al., 2010). Seeds are compressed ovoid or elliptical, been-like, 10-15cm long, dark brown, oily. Seeds contain 27.5% oil and 13.5% mucilage, also traces of an essential and a complex amino acid named glabrin. Karanjin and pongamol is non-glyceride portion of the oil and are major flavones of the seeds. However, antidiarrheal activity of seed extract of *Pongamia pinnata* had not been carried out so far, hence, this leads us to do study.

**KEYWORDS :** *Pongamia pinnata*, anti-diarrheal activity, castor oil

**MATERIALS AND METHODS**

**Plant Material**

Seeds of *Pongamia pinnata* were collected from the local area of Kopargoan, Maharashtra, India, in month of January and February and the Seeds were identified by Mr. P.S.N. Rao, Join Director, Botanical survey of India, Koregaon road, Pune. The herbarium of the plant specimen has been deposited at B.S.I. Pune, the voucher specimen No. being BRD1. Fresh plant material were washed under running tap water, air dried for two week and then homogenized to fine powder and stored in airtight bottles.

**Preparation of extract**

The powdered seeds of *Pongamia pinnata* were subjected to successive extraction process using soxhlet apparatus. Powder was packed into soxhlet column and extracted with Petroleum ether (60-80°C) for 24 hrs. The same marc was successively extracted with ethanol for 24 hrs. and afterwards with aqueous extract for 24 hrs. The extract was concentrated under reduced pressure. The dried extracts were stored in airtight container.

**Experimental animals**

Swiss albino rats (150-180 g) of either sex were selected for the experiments. Institutional Animal Ethics Committee approved the experimental protocol. Animals were housed in polypropylene cages. Animal handling was performed according to Good Laboratory Practice (GLP).

**Chemicals and Reagents**

Loperamide (Standard reference anti diarrhoeal drug), castor oil (Laxative agent) and vehicle (2% v/v Tween 80 in distilled water) were used.

**Phytochemical analysis of the extracts**

Phytochemical Screening of Plant materials the presence of saponins, tannins, carbohydrates, alkaloids, flavonoids glycosides, steroids, proteins and alkaloids, were detected by simple qualitative methods (Khandelwal, 2011).

**Acute toxicity study**

Acute toxicity study for the extract of *Pongamia Pinnata* seeds was carried out for determination of LD₅₀ in female albino mice (20-30gm) by adopting fixed dose method LD₅₀ study done for finding lethal or effective dose.

**Castor oil induced diarrhea**

Rats of either sex were fasted for 18h and divided into five groups of six animals per group. Castor oil at a dose of 1ml/animal for the induction of diarrhoea. (A) Thirty minutes after castor oil administration, the first group (control group) received vehicle (0.5%...
v/v Tween 80 in distilled water), second group received referenced drug loperamide (1mg/kg body weight) while third, fourth and fifth groups were given pet ether, ethanol and aqueous extract at doses of 30mg/kg, 250mg/kg, 100mg/kg body weight respectively by oral route.

Animals of all groups were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the wet and fecal material and number of defection was noted up to 6h in transparent metabolic cages with filter paper at the base. The total weight of faces were recorded within a period of 6h and compared with the control group.

Statistical analysis
The data were presented as mean ± S.E.M., and statistical significance between treatment and control groups was analyzed using one-way ANOVA where P<0.05 was considered statistically significant.

RESULT AND DISCUSSION
Karanja (Pongamia pinnata) an ancient plant described in Vedas. Samhita and in many Nighantu it is a very important medicinal plant. Ethnobotanically it is used in many diseases viz. Diabetes, diarrhea, skin diseases as scabies etc. It is described in Susruta Samhita in Prameha chikitsa and also in many nighantu. To promote the usage of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Nadkarni, 1954).

Pharmacognosy is the study of medicines derived from natural sources. Under this pharmacognosy, pharmacology and phytochemistry are necessary for authentication of crude drug. Research work was carried out to find the phytochemical constituents and its antidiabetic activity. For this dried seeds of Karanja were then and grinded to make fine powder of it, later its ethanolic extract was prepared in Soxhlet apparatus. The photochemical investigation (Table 1) of the various solvent extract of P. pinnata showed the petroleum ether extract to contain only alkaloids, and steroids in high concentrations.

Table 1. Phytochemical analysis of Pongamia pinnata Seeds

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Aquous Petroleum ether</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+= presence, –= absence

Castor oil induced diarrhea
The petroleum ether, aqueous and ethanol extracts was found to be effective against castor oil induced diarrhea on experimental rats at various doses of 30, 250, 100 mg/kg body weight has been shown in Table 1. In petroleum ether, ethanol and aqueous extract of Pongamia pinnata seeds significantly reduce the mean weight of faces when compared to control group. Experimental results reflect the activity is more pronounced in ethanol extract at dose 250 mg/kg and subsequently in aqueous extract at dose 100mg/kg and in pet ether extract at dose 30mg/kg.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Route</th>
<th>Mean weight of stools after 6hrs. treatment (g±S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (TWEEN 80)</td>
<td>5ml/kg oral</td>
<td>2.57±0.1411</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Std (Loperamide)</td>
<td>1mg/kg oral</td>
<td>0.50±0.060</td>
<td></td>
</tr>
</tbody>
</table>

Diarrhea is most commonly caused by gastrointestinal infections, which kill around 1.8 million people globally each year, mostly children in developing countries (WHO, 2004). The main cause of death from diarrhea is dehydration, which results from loss of electrolytes in diarrhoeal stools. Leaves of Pongamia pinnata (L.) Pierre (synonym, P. glabra vent) has been known as a remedy for diarrhea (Brijesh et al., 2006). Shoba and Thomas, 2001 reported on the effectiveness of P. pinnata in controlling castor oil induced diarrhea. While data are available on the effect of medicinal plants on intestinal motility and their antibacterial activity, there is a paucity of information on their mode of action on various aspects of diarrheal pathogenicity, namely colonization to intestinal epithelial cells and production/action of enterotoxins (Brijesh et al., 2006). Brijesh et al., (2006) evaluated the crude decoction of dried leaves of Pongamia pinnata for its antimicrobial (antibacterial, antigiardial and antitroviral) effect; and its effect on production and action of enterotoxins (cholera toxin, CT; Escherichia coli labile toxin, LT; and E. coli stable toxin, ST); and adherence of enteropathogenic E. coli and invasion of enteroinvasive E. coli and Shigella flexneri to epithelial cells. The decoction had no antibacterial, antigiardial and antitroviral activity, but it was found to reduce the production of CT and bacterial invasion to epithelial cells. These results indicated that the crude decoction of P. pinnata has selective antidiarrheal action with efficacy against cholera and enteroinvasive bacterial strains causing bloody diarrhea episodes. They attributed the antidiarrheal activity to antimotility, antisecretory and antimicrobial actions of the compound (Brijesh et al., 2006). 

CONCLUSION
This study provides the rationale for the use of the seed extracts of Pongamia pinnata as an antidiarrheal drug. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrheal activity observed.

REFERENCES